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Cerivastatin Triggers Tumor-specific Apoptosis with Higher Efficacy than Lovastatin[1]

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ABSTRACT

The statin family of drugs inhibits 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme of the mevalonate pathway, and is used clinically as a safe and effective approach in the control of hypercholesterolemia. We have shown previously (Dimitroulakos, J., Nohynek, D., Backway, K. L., Hedley, D. W., Yeger, H., Freedman, M. H., Minden, M D., and Penn, L. Z. Increased sensitivity of acute myelogenous leukemias to lovastatin-induced apoptosis: a potential therapeutic approach. Blood, *93:* 1308–1318, 1999) that lovastatin, a prototypic member of the statin family, can induce apoptosis of human acute myeloid leukemia (AML) cells in a sensitive and specific manner. In the present study, we evaluated the relative potency and mechanism of action of the newer synthetic statins, fluvastatin, atorvastatin, and cerivastatin, to trigger tumor-specific apoptosis. Cerivastatin is at least 10 times more potent than the other statins at inducing apoptosis in AML cell lines. Cerivastatin-induced apoptosis is reversible with the addition of the immediate product of the HMG-CoA reductase reaction, mevalonate, or with a distal product of the pathway, geranylgeranyl pyrophosphate. This suggests protein geranylgeranylation is an essential downstream component of the mevalonate pathway for cerivastatin similar to lovastatin-induced apoptosis. The enhanced potency of cerivastatin expands the number of AML patient samples as well as the types of malignancies, which respond to statin-induced apoptosis with acute sensitivity. Cells derived from acute lymphocytic leukemia are only weakly sensitive to lovastatin cytotoxicity but show robust response to cerivastatin. Importantly, cerivastatin is not cytotoxic to nontransformed human bone marrow progenitors. These results strongly support the further testing of cerivastatin as a novel anticancer therapeutic alone and in combination with other agents *in vivo*.

INTRODUCTION

The statin family of drugs targets HMG-CoA[6] reductase, the rate-limiting enzyme of the mevalonate pathway (1). The statin drugs are currently used widely as a safe and effective therapeutic in the treatment of hypercholesterolemia (2). We have reported previously (3) that one member of the statin family, lovastatin, triggers apoptosis in established and primary AML cells but not in normal bone marrow progenitors at doses that are achievable *in vivo*. Further support for the use of statins as a novel therapeutic in the treatment of cancer has been shown in retrospective studies (4) of statin trials where the incidence of cancer is decreased 28% in patients prescribed HMG-CoA reductase inhibitor therapy compared with those prescribed bile acids. Hence, the statin family may be a potential immediate therapeutic for the treatment of AML as well as other sensitive tumor types.

The statin family is composed of six members: pravastatin, simvastatin, lovastatin, fluvastatin, atorvastatin, and cerivastatin. The first three are derived from fungal fermentation, whereas the latter three are synthetic (2). Each member differs in its specificity for HMG-CoA reductase, purity, prodrug or active administrative form, and metabolism (5, 6). The fungal-derived agents have been shown to possess similar antiproliferative properties on AML-derived cells (3, 7, 8); however, the sensitivity and specificity of the synthetic statins to trigger AML-specific apoptosis remain unclear. Identifying the statin with maximal efficacy and specific activity will have direct impact on the clinical application of statin therapy in the management of cancer.

In this study, we compare the potency of the synthetic statins (fluvastatin, atorvastatin, and cerivastatin) to lovastatin (Fig. 1) for their ability to inhibit AML cell viability. Moreover, we explore

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[6] The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; AML, acute myeloid leukemia; FBS, fetal bovine serum; GGPP, geranylgeranyl pyrophosphate; FPP, farnesyl pyrophosphate; MTT, 3-4,5-dimethylthiazolyl-2,2,5-diphenyl tetrazolium bromide; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling; MTT50, the concentration required to reduce the MTT activity by 50%; MTT30, the concentration of drug required to reduce the MTT activity by 30%; FITC, fluoroscein isothiocyanate; ALL, acute lymphocytic leukemia.

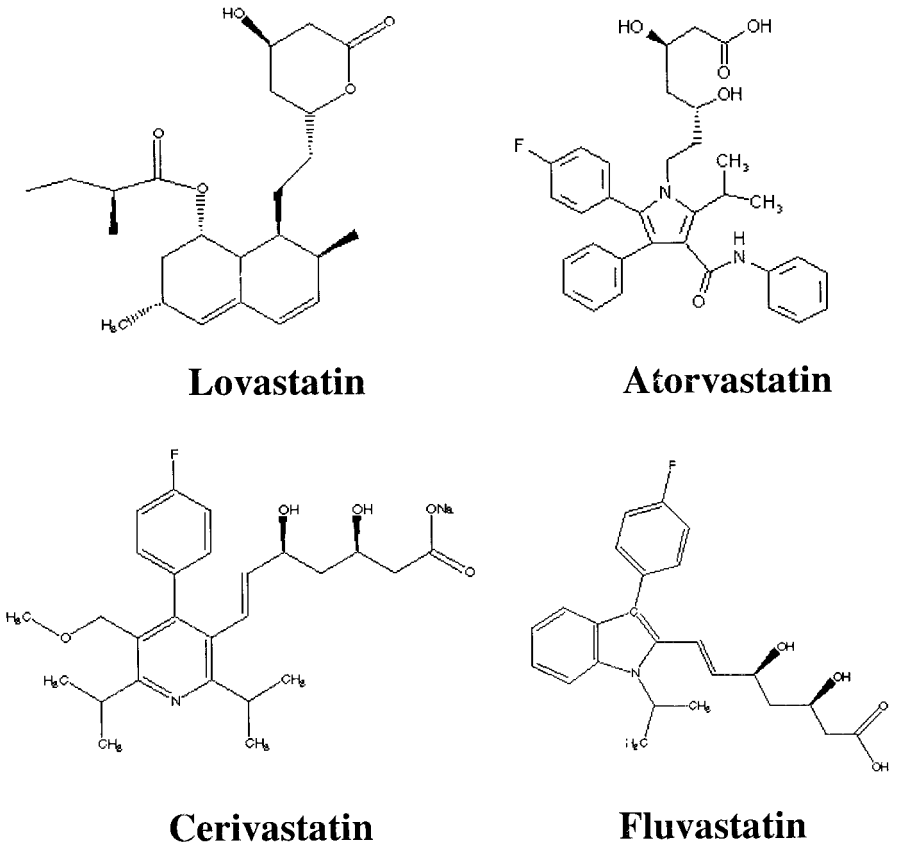

Fig. 1 Schematic diagram showing the structure of the statins under investigation in this study. Lovastatin is one of the prototypic inhibitors of HMG-CoA reductase and is derived from fungal fermentation, whereas atorvastatin, cerivastatin, and fluvastatin are newer synthetic inhibitors of this rate-limiting enzyme of the mevalonate pathway.

and compare the mechanism of action of lovastatin with the most potent statin, cerivastatin. We also determine that the increase in potency of cerivastatin to trigger apoptosis results in a greater number of statin-sensitive AML patient samples, expands the spectrum of tumors sensitive to statin therapy, and, importantly, retains tumor specificity.

MATERIALS AND METHODS

Cells and Cell Culture Conditions. AML cell lines OCI-AML-2, OCI-AML-3, OCI-AML-5 (hereafter referred to as AML-2, AML-3, and AML-5, respectively) and NB-4 and primary AML patient cells were all obtained from the Ontario Cancer Institute Leukemia Tissue Bank. The ALL cell lines B1, C1, and KK were derived as described previously (9) and obtained from Dr. M. Freedman (The Hospital for Sick Children, Toronto, Ontario, Canada). Cell lines were cultured in α-MEM (Princess Margaret Hospital Media Service, Toronto, Ontario, Canada), supplemented with 10% FBS and penicillin/streptomycin. Media for AML-5 cells was supplemented with 10% 5637 conditioned media.

Frozen patient cells were thawed and equilibrated overnight in α-MEM supplemented with 30% FBS, 10% 5637 conditioned media, and penicillin/streptomycin in a humidified incubator at 37°C and 5% CO_2. The next day, the concentration of FBS in the media was decreased to 20% for subsequent assays. Mononucleated cells were isolated from normal bone marrow using Ficoll-Hypaque and T-cell depleted. Peripheral blood blasts from presenting and relapsed patients and normal bone marrow from bone marrow transplant donors were collected after informed consent, according to institutional guidelines.

For dose-response experiments, cell lines and patient and normal bone marrow cells were exposed to increasing concentrations of statin (0 μM to 100 μM) for 48 h in a humidified incubator at 37°C and 5% CO_2. As solvent controls, ethanol and methanol were used for lovastatin and atorvastatin, respectively, whereas PBS was used for fluvastatin and cerivastatin. For the add-back experiments, cell lines were coincubated for 48 h with 1 μM cerivastatin and increasing concentrations of squalene (0 μM to 200 μM), mevalonate (0 μM to 300 μM), GGPP (0 μM to 10 μM), or FPP (0 μM to 10 μM).

The statins used in this study were kindly provided by their respective manufacturer. Lovastatin powder was a gift of Apotex Corp. (Mississauga, Ontario, Canada) and was activated as described previously (3, 10). Cerivastatin powder was a gift from Bayer Inc. (Wuppertal, Germany). Fluvastatin and atorvastatin powder were contributed by Novartis (Basel, Switzerland) and Parke-Davis (Ann Arbor, MI), respectively, and resuspended according to manufacturers' instructions. MTT, avidin-FITC, squalene, GGPP, and FPP were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Proliferation and Apoptosis Assays. The proliferation status and colony growth potential was determined using the MTT assay and the colony growth assay, respectively, as

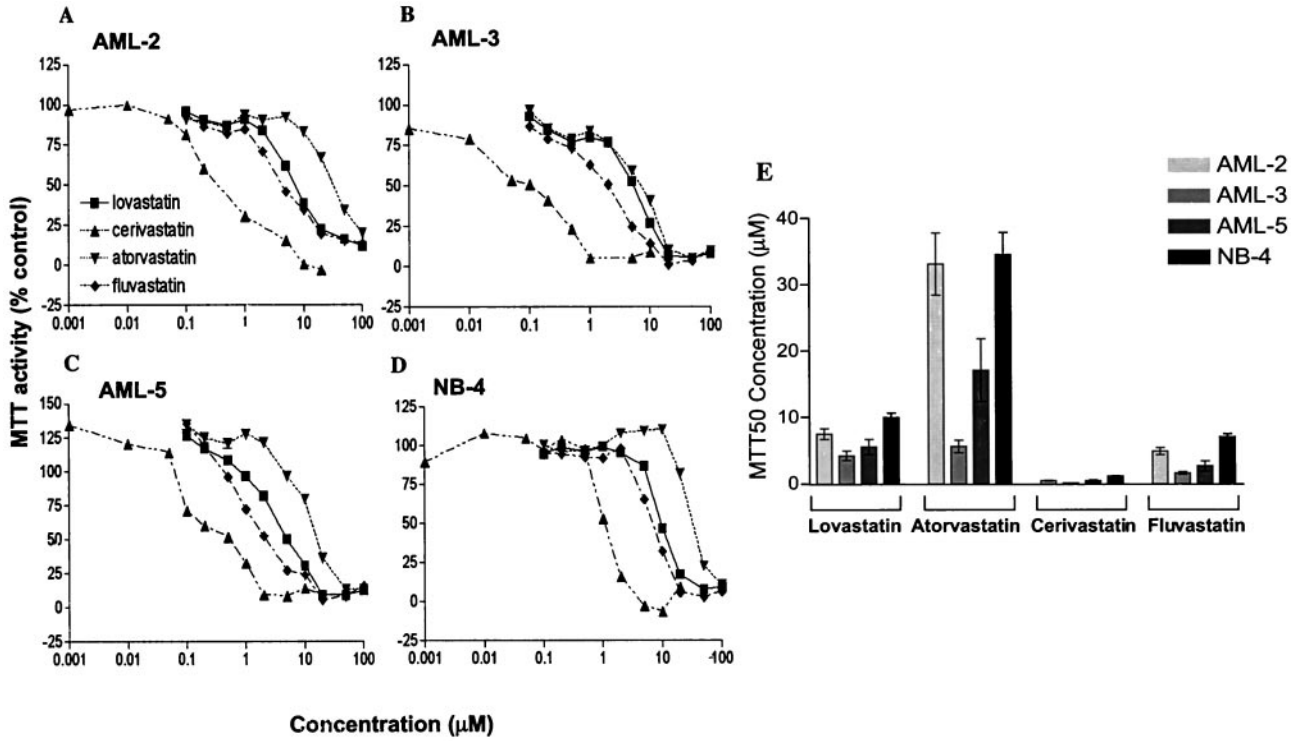

Fig. 2 MTT activity of AML cell lines after exposure to lovastatin, atorvastatin, fluvastatin, and cerivastatin. Cells were exposed to a wide concentration range (0 to 100 μM) of each statin for 48 h, and the effects on cell proliferation were determined using the MTT assay. MTT activity is plotted as a percentage of untreated controls and the mean and SE of three replicates in one experiment. Experiments were repeated three times with similar results, and a representative is shown. *A*, AML-2; *B*, AML-3; *C*, AML-5; *D*, NB-4; *E*, comparison of MTT50 of the four statins on all of the AML cell lines.

described in our previous manuscript (3). Apoptosis was determined using the TUNEL assay. For the TUNEL assay, the cells were exposed to drug for 48 h, harvested, and fixed by incubation in 4% formaldehyde for 15 min on ice. Subsequently, the cells were centrifuged, washed, resuspended in 70% ethanol, and stored at −20°C for up to 1 week. For analysis, 10^6 cells were incubated with 0.02 mM biotin-dUTP and 12.5 units of terminal deoxynucleotidyltransferase enzyme in a 1× reaction buffer [200 mM potassium cacodylate, 25 mM Tris-HCl, and 25 μg/ml BSA (pH 6.6)], 2.5 mM $CoCl_2$, and 0.01 mM dTTP (Roche Molecular Biochemicals, Laval, Quebec, Canada) for 45 min at 37°C. The cells were then washed once with PBS, labeled with avidin-FITC for 60 min at room temperature, washed again, and analyzed using a FACScalibur cytometer (Becton Dickinson, San Jose, CA).

RESULTS

AML-derived Cell Lines Show an Increased Sensitivity to Cerivastatin. To compare the sensitivity of AML cells to undergo apoptosis in response to lovastatin, fluvastatin, atorvastatin, and cerivastatin, we first used the MTT assay. MTT is a measure of the mitochondrial dehydrogenase activity within the cell and provides an indication of the proliferation status of the cells (11). The AML cell lines AML-2, AML-3, AML-5, and NB-4 were exposed to each statin over a wide concentration range (0 μM-100 μM) for 48 h. The MTT assay was then conducted, and the resulting absorbance values were normalized to untreated controls (Fig. 2). Representative MTT assays comparing the four statins in each cell line are shown in Fig. 2. Of the four statins tested, the AML cell lines were most sensitive to cerivastatin, followed by fluvastatin, lovastatin, and atorvastatin, as shown by the decrease in the MTT activity. A comparison of the MTT50s obtained for each cell line demonstrated that cerivastatin was the most potent statin tested in this assay (Fig. 2*E*).

Cerivastatin Induces Apoptosis by a Similar Mechanism to Lovastatin. We further examined the growth-inhibitory and cytotoxic effects of cerivastatin for mechanism of action. To ascertain whether the decrease in MTT activity in response to cerivastatin was attributable to the induction of apoptotic cell death, as seen previously (3) with lovastatin, we assayed the cells using the TUNEL assay. The TUNEL assay detects the DNA strand breaks induced during the process of apoptosis (12). Cerivastatin does indeed induce apoptosis in AML cells, as shown in the representative TUNEL profile (Fig. 3). The percentage of apoptotic cells is shown above the TUNEL-positive cells. AML-3 cells were exposed for 48 h to doses of lovastatin and cerivastatin, which reduced the MTT activity to approximately 10% compared with control. The ethanol control induced 2.3% apoptotic death, whereas 20 μM lovastatin induced 57.0% apoptotic death (Fig. 3, *A* and *B*, respectively). The PBS control for cerivastatin induced 2.6% apoptotic death, whereas 1 μM cerivastatin, a 20-fold lower

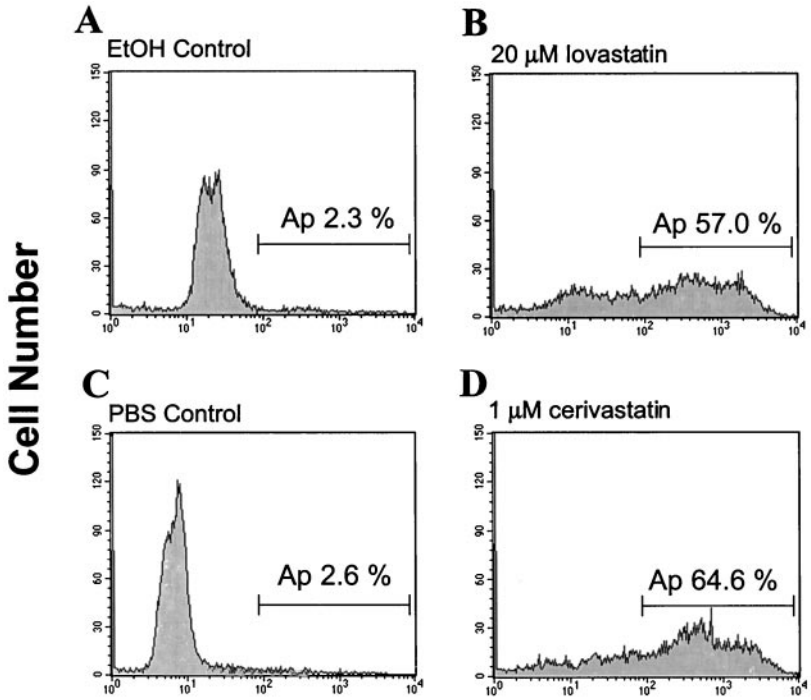

Fig. 3 Cerivastatin induces apoptosis of AML cell lines. Cells were exposed to *A*, ethanol; *B*, 20 μM lovastatin in ethanol; *C*, PBS; or *D*, 1 μM cerivastatin in PBS for 48 h, and induction of apoptosis was measured by the TUNEL assay. The percentage of TUNEL-positive cells is shown on the *right* side of each profile. Shown in this figure are representative profiles of AML-3 cells that have been assayed in three independent experiments. Similar results were obtained for the AML-2, AML-5, and NB-4 cell lines. *Ap*, apoptosis.

concentration than lovastatin, induced 64.6% apoptotic death (Fig. 3, *C* and *D*, respectively). Cerivastatin similarly induced elevated apoptotic death of AML-2, AML-5, and NB-4 cells, despite the concentration being 20-fold less than lovastatin (data not shown).

We have shown previously (10) that the inhibition of protein isoprenylation, in particular geranylgeranylation, holds a critical role in the mechanism of lovastatin-induced apoptosis in AML cells. To determine whether the mechanism of cerivastatin-induced apoptosis in AML cells was similar to lovastatin-induced apoptosis, we implemented an add-back approach to determine which products of the mevalonate pathway were critical to cerivastatin-induced apoptosis. Cells were coincubated with 1 μM cerivastatin and a range of concentrations of either mevalonate, squalene, GGPP, or FPP for 48 h and then assayed for indications of proliferation by the MTT assay (Fig. 4). The addition of mevalonate at 100 μM completely reversed the effect of 1 μM cerivastatin (Fig. 4*A*). Squalene, an intermediate in the cholesterol pathway, was added to the media at concentrations from 10 μM to 200 μM. No inhibition of cerivastatin-induced cytotoxicity was seen in any of the four AML cell lines with the addition of squalene (Fig. 4*B*). The addition of GGPP reversed the cytotoxic effects of 1 μM cerivastatin with a maximum effect (approximately 90% of control) at approximately 3 μM (Fig. 4*C*). The addition of FPP only partially reversed the effects of 1 μM cerivastatin with a maximum effect (approximately 20% of control) at a dose of approximately 2.5 μM (Fig. 4*D*). Hence, the mechanism of cerivastatin-induced apoptosis is similar to that of lovastatin, as reported previously (10).

Primary AML Patient Samples Which Weakly Respond to Lovastatin-induced Apoptosis Are Sensitive to Cerivastatin-induced Apoptosis. After observing the increased sensitivity of AML cell lines to cerivastatin relative to lovastatin, we wished to determine whether this increase in sensitivity was evident with primary AML patient material. We evaluated primary AML samples by the MTT and TUNEL assays after exposure for 48 h to various concentrations of cerivastatin. First, we examined whether patient material, which had been determined previously (3) as nonresponsive to lovastatin, would be sensitive or insensitive to cerivastatin. AML patient samples were defined previously (3) as nonresponsive when the MTT50 and MTT30 were greater than 100 μM. Analysis of two nonresponsive AML patient samples showed that neither patient sample was sensitive to lovastatin or cerivastatin (Fig. 5*A*). The MTT activity in both cases decreased to approximately 60–70%. Our previous work (3) indicates that a decrease in MTT activity similar to Fig. 5*A* corresponds to a nonapoptotic response. Indeed, analysis by the TUNEL assay revealed apoptosis did not occur when the patient cells were exposed to 150 μM lovastatin or 20 μM cerivastatin for 48 h (data not shown).

To determine whether AML patient samples that showed a weak response to lovastatin would respond to cerivastatin with an increase in sensitivity, we exposed AML patient samples that exhibited a weak response to lovastatin to cerivastatin for 48 h. The AML patient samples were then assayed by the MTT and TUNEL method. A weak response was defined previously (3) as samples with an MTT50 less than 100 μM and an MTT30 greater than 100 μM, whereas primary patient samples with both MTT50 and MTT30 values less than 100 μM were considered sensitive. Using the MTT assay, primary AML patient samples, which were weakly responsive to lovastatin, were significantly more sensitive to cerivastatin (Fig. 5*B*). Comparative analysis demonstrates that the MTT50 concentrations for patient samples exposed to cerivastatin were at least 10 times lower than the MTT50 concentrations for the same patient samples exposed to

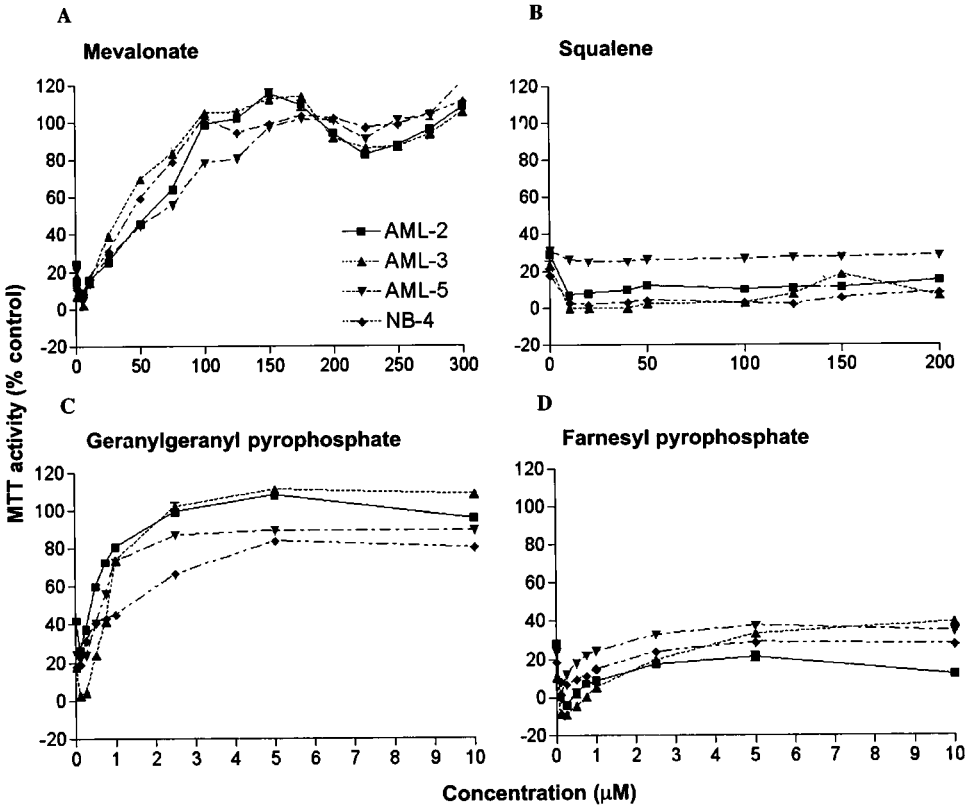

Fig. 4 Cerivastatin-induced apoptosis of AML cells is inhibited by mevalonate or GGPP. Cells were exposed to 1 μM cerivastatin and increasing concentrations of *A*, mevalonate; *B*, squalene; *C*, GGPP; or *D*, FPP for 48 h. Mitochondrial dehydrogenase activity was determined using the MTT assay. MTT activity is plotted as a percentage of untreated control and the mean and SE of three replicates in one experiment. Experiments were repeated three times with similar results.

lovastatin. This was a statistically significant difference ($P < 0.01$, using a paired *t* test; Fig. 5*C*). Similarly, when primary AML patient cells (patient D) were exposed to 100 μM lovastatin for 48 h, only 9.1% apoptotic death was detected by the TUNEL assay. By contrast, when the same primary AML patient cells were exposed to a 10-fold lower concentration of cerivastatin (10 μM), 24.8% apoptotic death was detected (Fig. 5*D*). Thus, a patient sample that is weakly responsive to lovastatin-induced apoptosis can be sensitive to the apoptotic effects of cerivastatin, whereas nonresponsive patient cells remain apoptosis-resistant across a large dose range of either lovastatin or cerivastatin.

ALL Cell Lines, Weakly Responsive to Lovastatin, Are Sensitive to Cerivastatin. The increased potency of cerivastatin suggested that the range of hematological malignancies sensitive to statin-induced apoptosis may be expanded. Previously (3), we showed that acute lymphocytic leukemic (ALL) cell lines were only weakly responsive to lovastatin-induced apoptosis. To determine whether the ALL cell lines may be sensitive to cerivastatin-induced apoptosis, we compared the response of three ALL cell lines (B1, C1, and KK) with a wide concentration range of lovastatin and cerivastatin using the MTT assay. By this screening approach, the ALL cell lines were more sensitive to cerivastatin (data not shown). Using the TUNEL assay, we determined that a greater number of ALL cells was indeed dying by apoptosis after 48 h exposure to cerivastatin compared with lovastatin at 20 μM, the dose at which we had shown previously (3) ALL cell lines lacked a significant apoptotic response. Representative TUNEL profiles of C1 cells after exposure to ethanol control, 20 μM lovastatin, PBS control, or 20 μM cerivastatin for 48 h are shown in Fig. 6*A*. After exposure to lovastatin for 48 h, the percentage of apoptotic cells was 6.9%, 4.4%, and 2.4% for B1, C1, and KK, respectively, compared with ethanol controls, 4.1%, 2.1%, and 2.4%, respectively (Fig. 6*B*). By contrast, exposure at the same concentration of cerivastatin induced 20.2%, 53.1%, and 20.1% apoptotic death in B1, C1, and KK cells, respectively, compared with PBS controls at 5.0%, 3.8% and 2.7%, respectively (Fig. 6*B*).

Cerivastatin Triggers Tumor-specific Apoptosis. The work of ourselves and others (3, 7, 8) has shown that HMG-CoA reductase inhibitors such as lovastatin have negligible effects on normal bone marrow progenitors and hematopoiesis. It remained unclear whether the elevated potency of cerivastatin to trigger tumor cell apoptosis would have an effect on normal bone marrow progenitors. To this end, we exposed normal bone marrow to lovastatin and cerivastatin at a range of concentrations. The normal bone marrow were then assayed using the MTT, colony growth, and TUNEL assays. After 48 h exposure to high concentrations (200 μM) of cerivastatin, normal bone marrow MTT activity was reduced to only 50% and was comparable with the effect of lovastatin (Fig. 7*A*). To determine the potential of progenitor cells to repopulate the hematopoietic system after exposure to cerivastatin for 48 h, the colony growth assay was used (13, 14). The normal bone marrow was exposed

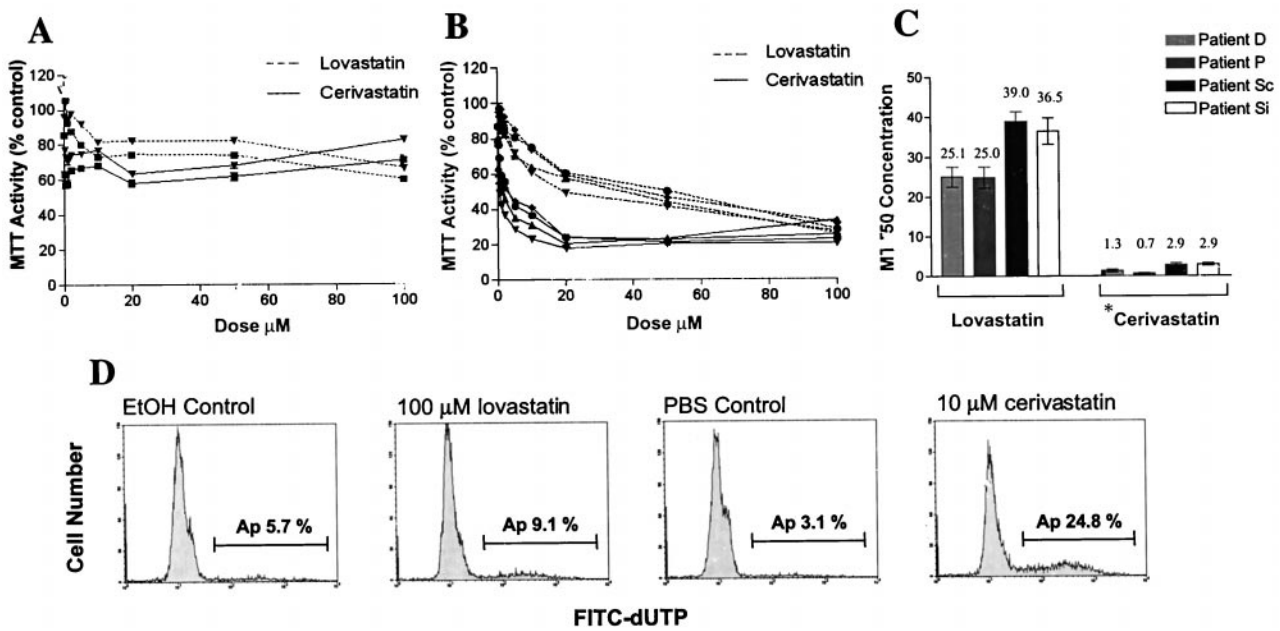

Fig. 5 Cerivastatin is a potent inducer of apoptosis in primary AML cells. Cells were exposed to increasing concentrations of lovastatin or cerivastatin (0 to 100 μM) for 48 h, and the effects on cell proliferation were determined using the MTT assay. MTT activity is plotted as a percentage of the untreated control and the mean and SD of three replicates in one experiment. This experiment was repeated in duplicate, with similar results. A representative is shown. *A*, patient G (■) and patient H (▼); *B*, patient D (■), patient P (▲), patient Sc (▼), and patient Si (◆). *C*, MTT50, the concentration of lovastatin and cerivastatin required to reduce the MTT activity by 50%, is plotted. Comparison of the MTT50 values for lovastatin and cerivastatin shows there is a statistically significant difference ($P < 0.01$). *D*, TUNEL histogram of AML patient D cells after exposure to 100 μM lovastatin or 10 μM cerivastatin for 48 h showing the induction of apoptosis. These results are representative of other patient samples similarly analyzed. *Ap*, apoptosis.

to 20 μM lovastatin and 1, 5, and 20 μM concentrations of cerivastatin for 48 h and then plated for colony growth assays. Colony formation of myeloid progenitor cells was comparable after exposure to solvent controls, 20 μM lovastatin, or 20 μM cerivastatin was seen (Fig. 7*B*). The TUNEL assay further revealed that after exposure to the doses used in the colony growth assay, 8.9% apoptotic death in normal bone marrow after exposure to 20 μM lovastatin and 1 μM cerivastatin compared with ethanol and PBS controls at 6.8% and 5.1% (Fig. 7*C*). We determined that the effect of cerivastatin on normal bone marrow was negligible and similar to lovastatin.

DISCUSSION

The statin family of drugs inhibits the mevalonate pathway by targeting HMG-CoA reductase and is the drug of choice for treating hypercholesterolemia. We and others have shown previously (3, 8, 15–17) that statins can trigger tumor cells derived from certain cancers, including AML, to undergo apoptosis *in vitro* and reduce tumor load *in vivo*, suggesting statins may hold a role as a novel therapeutic in the clinical management of cancer. To fully evaluate the safety and efficacy of statins in anticancer clinical trials, it is difficult to know which of the many statins will be the drug of choice. To this end, we set out to determine which statin shows the highest specific activity to trigger tumor-specific cell death *in vitro*. In this study, we compared the most recent generations of synthetic statins, fluvastatin, atorvastatin, and cerivastatin, to one of the original fungal-derived statins used in our previous studies, lovastatin. We showed that all of the four statins were cytotoxic to AML cell lines in a dose-dependent manner. Cerivastatin consistently showed at least a 10-fold higher efficacy in triggering AML cell death compared with the other statins. Mechanistically, we show cerivastatin killed AML cells in a similar manner to lovastatin. Cell death occurred by an apoptotic mechanism and was blocked by mevalonate, the immediate downstream product of HMG-CoA reductase, as well as an end product of the pathway, GGPP. Importantly, we also show the specificity of the apoptotic response is restricted to transformed cells because normal bone marrow progenitors were refractory to cerivastatin as well as lovastatin-induced death. Moreover, we show the elevated efficacy of cerivastatin-induced apoptosis increases both the number and type of malignancies that are sensitive to statin cytotoxicity.

Our previous analysis (3) of primary AML patient samples showed the majority was sensitive to lovastatin-induced apoptosis. However, two patient AML samples were nonresponsive to lovastatin exposure. Despite the increased efficacy of cerivastatin, these two samples remain refractory to apoptosis induction. This data further reinforces that both cerivastatin and lovastatin use a similar mechanism of action. Moreover, this shows that mechanisms of drug resistance to statin cytotoxicity exist and that these may not necessarily be overcome by the increased potency afforded by cerivastatin. Interestingly, in our previous study (3) of AML patient samples, a subset was only

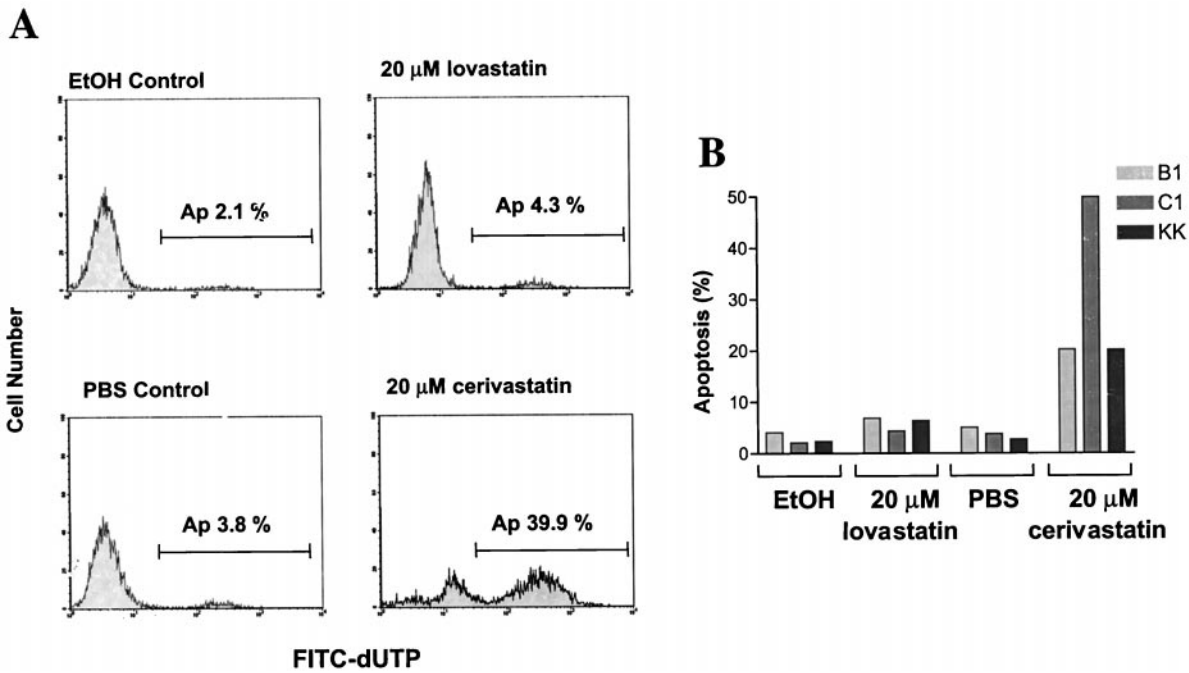

Fig. 6 Cerivastatin induces apoptosis of ALL cells. *A*, TUNEL profiles of ALL C1 cells exposed to ethanol control, 20 μM lovastatin, PBS control, or 20 μM cerivastatin for 48 h showing the increase the percentage of cells undergoing apoptosis after treatment with cerivastatin. *B*, histogram showing the percentage of apoptotic cells as determined by the TUNEL assay in the ALL cell lines (B1, C1, and KK) after 48 h exposure to solvent controls, 20 μM lovastatin, or 20 μM cerivastatin. These results are representative of two independent experiments.

weakly sensitive to lovastatin and exhibited an apoptotic response only after prolonged exposure or after treatment with elevated nonphysiological doses of drug. By contrast, exposure to cerivastatin revealed these weakly sensitive AML patient cells could be killed more rapidly and at a lower dose, suggesting these additional patients may be sensitive to the anticancer therapeutic potential of cerivastatin. As well as increasing the number of sensitive AML patient samples, the elevated efficacy of cerivastatin has similarly enabled additional hematological malignancies to be included in the growing list of statin-sensitive tumor types. Cell lines derived from ALL patients were shown previously (3) to be only weakly sensitive to lovastatin-induced apoptosis, requiring exposure to doses of 20 μM for approximately 4 days to detect a cytotoxic response. By contrast, exposure to cerivastatin shows ALL cell lines can undergo apoptosis in a sensitive manner *in vitro*. These results indicate: (*a*) a larger number of patients may respond to cerivastatin treatment; and (*b*) the range of tumor types sensitive to statin therapy may be expanded with the use of cerivastatin. It will be informative to extend these observations and determine the other tumor types that are sensitive to cerivastatin-induced apoptosis. It will likely include and extend the list of tumor types we and others have shown previously (18–22) to be sensitive to lovastatin. This information will be instructive for the design of clinical trials to evaluate the therapeutic efficacy of cerivastatin as an anticancer agent.

The wealth of data available from the extended use of statins in the control of cholesterol also suggests cerivastatin is the statin of choice for the cancer clinic. Like other statins, cerivastatin has been shown to have negligible adverse side effects at the therapeutic dose for cholesterol. The maximum dose of cerivastatin reported to date is 0.8 mg/day for a 4-week trial with adverse effects being no greater than placebo control (23, 24). Contraindications for the statins are few (rash, nausea, fever, myopathy, and liver toxicity) but are dose-related and can be avoided by monitoring transaminase and creatine kinase levels, since elevation in these enzymes has been associated with adverse side effects (24, 25). Importantly, clinical experience shows any adverse side effects are reversible when statin administration is terminated, further emphasizing the relative safety of these agents as a novel therapeutic for the cancer clinic. The pharmacokinetics of cerivastatin is far superior to that of lovastatin, further suggesting it is the agent of choice for future antitumor therapeutic studies. First, cerivastatin is enantiomerically pure and is in an active β-hydroxy acid form, whereas lovastatin is administered as a lactone prodrug form and is dependent upon first-pass metabolism for activation (26–28). Comparative bioavailability of cerivastatin is 60%, whereas lovastatin is approximately 5% (5, 29). In addition, unlike all of the other statins, cerivastatin possesses high systemic bioavailability attributable to little first-pass extraction. Moreover, there is extensive patient-to-patient variability in lovastatin metabolism because it is dependent upon the activity of cytochrome P450 3A4, which is commonly affected by other drugs and food intake (5, 30). By contrast, cerivastatin biotransformation is processed by two independent enzymes, cytochrome P450 2C8 as well as 3A4 (26, 29, 31). Indeed, cerivastatin metabolism is not influenced by rivastatin, warfarin, antacid, cimetidine, cho-

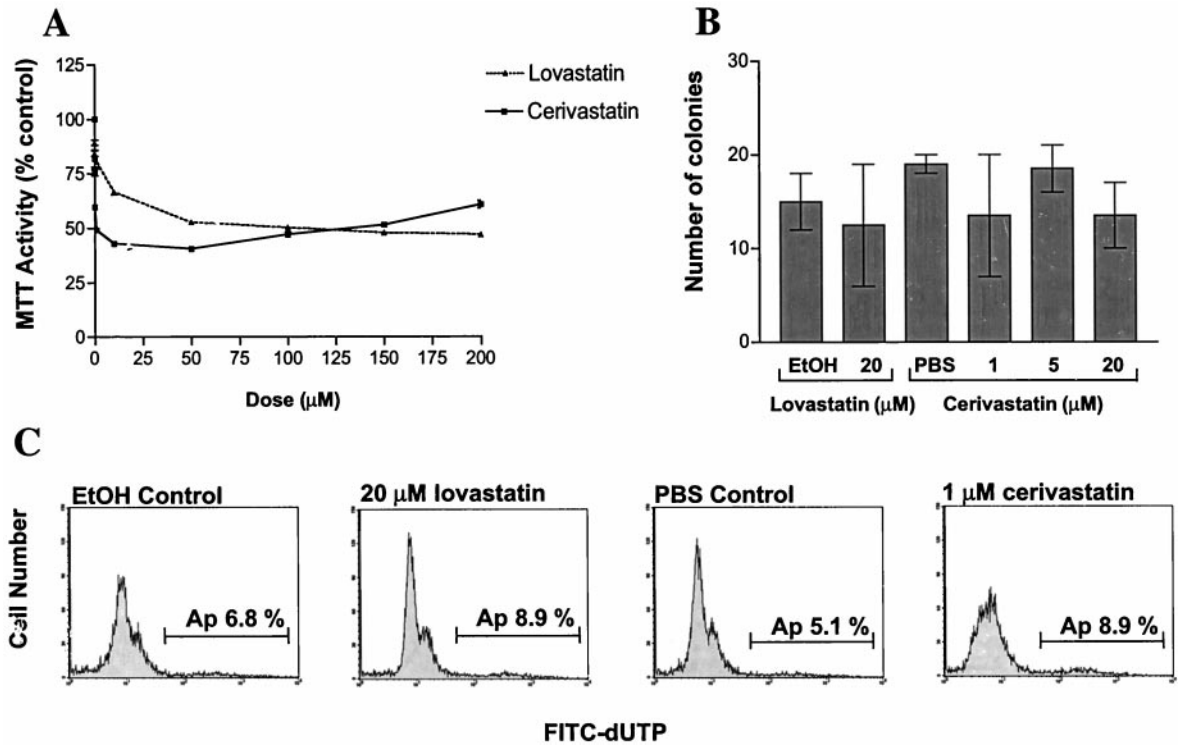

Fig. 7 Normal bone marrow cells are not sensitive to cerivastatin-induced apoptosis. Sensitivity to cerivastatin was tested by the MTT assay (*A*), colony growth assay (*B*), and the TUNEL assay (*C*). To assess possible antiproliferative effects over a broad dose range, cells were exposed to 0.01 to 200 μM lovastatin and cerivastatin for 48 h and analyzed for MTT activity. To evaluate sensitivity of progenitors, cells were plated in methylcellulose after 48 h of exposure to ethanol control, 20 μM lovastatin, PBS control, 1 μM, 5 μM, or 20 μM cerivastatin and incubated for 14 days. To measure apoptosis, cells were incubated with ethanol control, 20 μM lovastatin, PBS control, or 1 μM cerivastatin for 48 h, and induction of apoptosis was determined by the TUNEL assay. Shown are representative results of three independent MTT, colony growth, and TUNEL assay experiments.

lestyramine, erythromycin, or digoxin, which can influence the efficacy and safety of other statins when used in combination therapy for hyperlipidemia (26, 32–35). That cerivastatin is less subject to drug-drug interactions further strengthens its potential role as an antitumor agent since cancer therapy is often administered as a cocktail of agents.

It will be of interest to determine whether cerivastatin will synergize with other AML chemotherapeutics, as has been shown previously for lovastatin; *e.g.*, Feleszko *et al.* (36) have shown synergy between lovastatin and doxorubicin in mouse tumor models. Cerivastatin will likely exhibit similar synergistic effects as lovastatin, as we have shown these two agents share a similar mechanism of action. Indeed, understanding the mechanism of action of statin-induced apoptosis has two immediate clinical applications. First, synergistic interactions between statins and different therapeutic options can be better predicted because statins appear to trigger apoptosis by blocking isoprenylation of proteins by depleting GGPP pools. Second, geranylgeranylation of protein substrates can be used as an *in vivo* biomarker of statin activity in patient tumor samples during clinical trials to determine whether the drug is directly affecting the target cell by the proposed mechanism. It will be important to determine the maximum tolerated dose of lovastatin and cerivastatin and then determine whether these agents can affect tumor load. Our experience to date suggests there is a role for statins in the management of cancer, since we reported recently (17) a case study of AML blasts controlled by lovastatin in an elderly AML patient.

Clearly, statins can do more than simply lower cholesterol. Our studies suggest the statin family of drugs should be evaluated for their safety and efficacy alone and in combination with other agents as a novel therapeutic option for the treatment of cancer. We suggest certain cancers are more sensitive than others to statin therapy (3, 22) and that the clinical and pharmacological characteristics of cerivastatin make cerivastatin the statin of choice for this new application. The safety profile of this family of drugs and the fact that they are readily available simplify the testing of these drugs in the clinical management of cancer.

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